

Commissioner of Patents
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AMENDMENTS TO THE SPECIFICATION

Please replace paragraphs [00107], [00109], [00114], [00118], [00120], [00123], [00135], [00139], [00188], [00202], [00293], [00299] and [00300] with the following amended paragraphs:

[00107] Figure 1. Plaque reduction assay conducted in VERO cells using HSV-1 (strain KOS). Infected cells are treated with increasing concentrations of REP 1001 (SEQ ID NO: 1; a), REP 2001 (SEQ ID NO: 2; b) or REP 3007 (SEQ ID NO: 3; c). IC₅₀ values calculated from linear regressions are reported in each graph.

[00109] Figure 3. Plaque reduction assay conducted in VERO cells using HSV-1 (strain KOS). Infected cells are treated with increasing concentrations of REP 2001 (SEQ ID NO: 2; a), REP 2002 (b) or REP 2003 (c), REP 2004 (d), REP 2005 (e), REP 2006 (f) and Acyclovir (g). IC₅₀ values calculated from linear regressions are reported in each graph.

[00114] Figure 8. Plaque reduction assay conducted in VERO cells using HSV-1 (strain KOS). Unmodified ODNs, PS-ODNs with a random sequence and PS-ODNs targeting the start codon of HSV-1 IE110 were tested in increasing concentrations. REP 2013 (a), REP 2014 (b), REP 2015 (c), REP 2016 (SEQ ID NO: 4; d), REP 2017 (SEQ ID NO: 5; e), REP 2018 (SEQ ID NO: 6; f), REP 2019 (SEQ ID NO: 19; g), REP 2020 (SEQ ID NO: 8; h) and REP 2021 (SEQ ID NO: 9; i). IC₅₀ values calculated from linear regressions are reported in each graph.

[00118] Figure 12. Plaque reduction assay conducted in human fibroblast cells using HSV-2 (strain MS2). Infected cells are treated with increasing concentrations of REP 1001 (SEQ ID NO: 1;

a), REP 2001 (SEQ ID NO: 2; b) or REP 3007 (SEQ ID NO: 3; c). IC_{50} values calculated from linear regressions are reported in each graph.

[00120] Figure 14. Plaque reduction assay conducted in VERO cells using HSV-2 (strain MS2). Infected cells are treated with increasing concentrations of REP 2001 (SEQ ID NO: 2; a), REP 2002 (b) or REP 2003 (c), REP 2004 (d), REP 2005 (e), REP 2006 (f) and acyclovir (g). IC_{50} values calculated from linear regressions are reported in each graph.

[00123] Figure 17. Plaque reduction assay conducted in VERO cells using CMV (strain AD169). Three clinical CMV therapies were tested: Gancyclovir (a), Foscarnet (b) and Cidofovir (c). A broad range of PS-ODN randomizer sizes were also tested in increasing concentrations; REP 2003 (d), REP 2004 (e), REP 2006 (f) and REP 2007 (g). Finally, REP 2036 (Vitravene) was tested as synthesized in house (SEQ ID NO: 10; h) and as commercially available (SEQ ID NO: 11; i). IC_{50} values calculated from linear regressions are reported in each graph.

[00135] Figure 29. Determination of viral lysate binding to baits of different sizes by fluorescence polarization. REP 2032-FL (N6), REP 2003-FL and REP 2004-FL were tested for lysate binding in lysates from HSV-1 (a), HIV-1 (b) or RSV (c).

[00139] Figure 33. The ability of double stranded PS-ODNs to bind to viral lysates is tested by fluorescence polarization. Single stranded (ss) or double stranded (ds) phosphorothioated REP 2017 (SEQ ID NO: 5; fluorescently labeled) was prepared as well as its non-thioated analog (2017U). These baits were tested for binding to HSV-1 and HIV-1 viral lysates.

[00188] A random sequence (REP 2017; SEQ ID NO: 5) and its complement (either PS modified or unmodified) are fluorescently labeled as described elsewhere and tested for their ability to bind to purified HSV-1 and HIV-1 proteins by fluorescence polarization as described in the present invention. Hybridization was verified by acrylamide gel electrophoresis. Unmodified REP 2017 (2017U; SEQ ID NO: 5), either single (ss) or double stranded (ds), had no binding activity in either HSV-1 or HIV-1 lysates. However, PS modified REP 2017 (SEQ ID NO: 5), either single stranded or double stranded, was capable of HSV-1 and HIV-1 interaction (see figure 33).

[00202] The ability of a range of sizes of PS-ODN randomers to bind to these proteins was also tested using fluorescent versions of REP 2032 (N6), REP 2003, REP 2004, REP 2006 and REP 2007 (see figure 32). We observe that for p24, there is no size dependent interaction with p24 (see figure 32a) however; we did see an increase in gp41 binding in PS-ODN randomers larger than 20 bases versus those less than 20 bases (see figure 32b). This suggest when PS-ODN randomer length increases above 20 bases, multiple copies of gp41 can bind to individual randomers, increasing their polarization.

[00293] To test if PS-ODNs could inhibit HSV-1, REP 1001 (SEQ ID NO: 1), 2001 (SEQ ID NO: 2) and 3007 (SEQ ID NO: 3) are tested in the HSV-1 PRA. It is expected that only REP 2001 (SEQ ID NO: 2) will show any activity as this PS-ODN is directed against the origin of replication in HSV (the other two are directed against replication origins in humans and plasmids). However all three PS-ODNs showed anti-HSV-1 activity (see FIG. 1). Moreover, the potency of the anti-HSV-1 effect is dependent on the size of the oligo (see figure 2).

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[00299] To test if PS-ODNs could inhibit HSV-2, REP 1001 (SEQ ID NO: 1), 2001 (SEQ ID NO: 2) and 3007 (SEQ ID NO: 3) are tested in the HSV-2 PRA. It is expected that only REP 2001 (SEQ ID NO: 2) will show any activity as this PS-ODN is directed against the origin of replication in HSV-1/2 (the other two are directed against replication origins in humans and plasmids), however all three PS-ODNs showed anti-HSV-2 activity (see figure 12). Moreover, the potency of the anti-HSV-2 effect is dependent on the size of the PS-ODN and independent of the sequence (see figure 13).

[00300] To confirm the size dependence and sequence independence of PS-ODNs on anti-HSV-2 activity, we test PS-ODNs that vary in size (REP 2002, 2003, 2004, 2005 and 2006). These PS-ODNs are rendered inert with respect to sequence specific effects by synthesizing each base as a "wobble" (N) so that each PS-ODN actually represents a population of different random sequences with the same size, these PS-ODNs are termed "randomers". When these PS-ODNs are tested in the HSV-2 PRA, we find that PS-ODNs 10 bases or lower had no detectable anti-HSV-2 activity but as the size of the PS-ODN increases above 10 bases, the potency also increases (IC_{50} decreases, see figure 14 and 15). We also noted that PS-ODNs greater than 20 bases had IC_{50} values significantly lower than a clinically accepted anti-HSV-2 drug, acyclovirTM (see figure 15).